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# **Occurrence of Enzyme Systems for Production and Decomposition of Oxalate in a White-Rot Fungus *Coriolus versicolor* and Some Characteristics of Glyoxylate Oxidase\*<sup>1</sup>**

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## **Introduction**

Oxalic acid occurring in higher plants, cultures of molds and wood-rotting fungi, has been receiving much attention from various viewpoints<sup>1)</sup>. Several higher plants, including *Oxalis* spp., seem to accumulate oxalic acid in leaves to protect themselves against fungal attack. Plant pathogens produce oxalic acid to attack crops<sup>2)</sup>. Symbiotic mycorrhizal fungi seem to excrete the acid to liberate phosphorus from calcium phosphate and other minerals so that host plants utilize them for their growth<sup>3)</sup>. Symbiotic rhizobia also biosynthesize oxalic acid, which is used as an electron donor for nitrogen fixation in broad beans (*Vicia faba*)<sup>4)</sup>. Recently, Akamatsu and Shimada partially purified glyoxylate oxidase, which catalyzes the oxidation of glyoxylate to form oxalate, from brown-rot basidiomycete *Tyromyces palustris*<sup>5)</sup>. However, the oxalate producing enzyme from white-rot fungi has not yet been characterized, although the enzyme was obtained in cell-free extracts<sup>6,7)</sup>. We report here occurrence of glyoxylate oxidase, formate dehydrogenase, and also oxalate decarboxylase in the white-rot fungus *Coriolus versicolor* and some characteristics of the glyoxylate oxidase.

## **Materials and Methods**

*Coriolus versicolor* (COV-1030; kindly supplied from Laboratory of Deterioration Control, Wood Research Institute, Kyoto University) was grown, the mycelia were

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harvested on day 21 of the cultivation, and glyoxylate oxidase was extracted as described previously<sup>6)</sup>. The enzyme proteins were precipitated by addition of ammonium sulfate to 80% saturation, the precipitate was collected, dialyzed, and the enzyme solution was used as a crude preparation.

The reaction mixtures (3 ml each) contained glyoxylate substrate (20 mM, pH 4.0), 12  $\mu$ l of 5 mM DCIP, 0.5 ml of the crude enzyme solution, 1 ml of 0.1 M borate buffer (pH 8.0) and distilled water. The initial velocity of the reduction of DCIP was determined by measurement of the decrease in absorbance at 600 nm ( $\epsilon = 17.2 \text{ mM}^{-1} \text{ cm}^{-1}$  at pH 8.0) at 40°C<sup>5)</sup>.

Oxalate decarboxylase was assayed at pH 4.0 according to the method of Dutton *et al.*<sup>8)</sup>. Formate dehydrogenase was assayed by measurement of the increase in absorbance at 340 nm according to the reported method<sup>9)</sup> except for the use of 67 mM of dipotassium hydrogen phosphate buffer pH 9.5.

Alternatively, for identification of the oxalate product formed from glyoxylate, the product was confirmed by GC-MS analysis of the amide derivative of the product<sup>10)</sup> and determined by the assay of oxalate product with the commercial enzyme kits for oxalate as reported previously<sup>6,7)</sup>.

## Results and Discussion

*Changes in activities of glyoxylate oxidase, oxalate decarboxylase, and formate dehydrogenase in Coriolus versicolor:* Activities of oxalate producing enzyme (glyoxylate oxidase) and oxalate-decomposing enzymes (oxalate decarboxylase and formate dehydrogenase) and the amounts of oxalate produced during the cultivating of the white-rot fungus were assayed. The results are shown in Fig. 1. The increase in activities of both glyoxylate oxidase and oxalate decarboxylase are in good harmony with the changes in production of oxalate. Formate dehydrogenase reached the maximum on day 14 and decreased. The reason for the lower activity of formate dehydrogenase is not clear. However, excess oxalate may be decomposed predominantly by lignin peroxidase systems<sup>11)</sup> rather than formate dehydrogenase.

*Optimal pH and temperature:* The glyoxylate oxidase exhibited the maximum activity at pH 9 (borate-potassium chloride buffer).

*Substrate specificity:* The relative activities of glyoxylate oxidase for various substrates were compared. The results indicate that both glyoxylate and glycolaldehyde were the best substrates among the compounds tested. The activity for glycollate was half of that for glyoxylate, but no other compounds such as glyoxal, acetaldehyde, formaldehyde, formate, oxalate and L-malate were found to be the substrate. It is noteworthy that the glyoxylate oxidase from *T. palustris*<sup>5)</sup> utilized glyoxylate substrate best but have little activity for

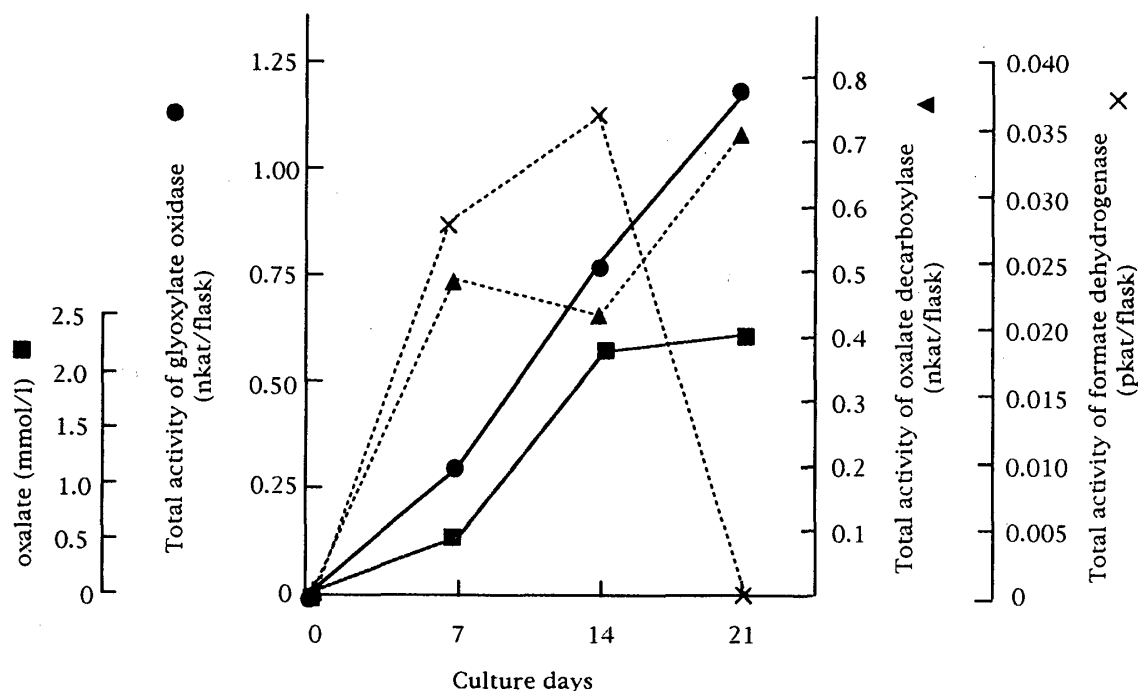


Fig. 1. Changes in activity of the oxalate-producing and -decomposing enzymes during the cultivation of *C. versicolor*.

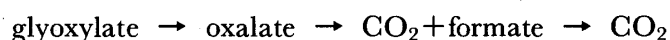
glycolaldehyde and glycollate in contrast to the enzyme from *C. versicolor*.

**Effects of inhibitors:** Although metal ion chelators such as EDTA, thiourea and azide anions as heme-enzyme inhibitor did not inhibit the enzyme. However, Tiron, which is one of metal ion (iron) chelators, inhibited half of the activity. However, *p*-chloromercuribenzoate (*p*CMB) was found to inhibit the activity completely, which indicates the sulphhydryl group is involved in the catalytic function of the enzyme. The preincubation of the enzyme with 1mM hydroxylamine according to the Shinagawa *et al.*<sup>12)</sup>, did not inactivate the enzyme. The result indicates that PQQ (pyrroloquinoline quinone)-enzyme is not involved in the oxidation of glyoxylate, because PQQ has been reported to be inhibited by  $\text{NH}_2\text{OH}$ <sup>12)</sup>. Interestingly, oxalate was found to potently inhibit glyoxylate oxidase activity. The overproduction of oxalate within the cellular sites may be controlled by the oxalate itself in a manner product inhibition.

**Effectiveness of electron acceptor:** The effectiveness of electron acceptors for the enzymatic oxidation of glyoxylate was tested. The results indicate that DCIP was the second best electron acceptor after potassium ferricyanide, which was consisted with the observation for glyoxylate oxidase from *T. palustris*. Neither NAD nor NADP was effective, regardless of the presence or absence of CoA which has been reported to be necessary for glyoxylate dehydrogenase<sup>13)</sup>. The addition of the FMN or FAD did not enhance the activity, indicating that the enzymatic reaction was independent on the external addition of these

flavin nucleotides. However, it is not ruled out from this experiment alone the glyoxylate oxidase does not contain these flavin nucleotides as a prosthetic group, because these nucleotides are known to be frequently tightly bound to a variety of flavoprotein oxidases. In order to confirm the natural electron acceptor, further study must be carried out with the purified enzyme.

In conclusion : (1) The glyoxylate oxidase from the white-rot fungus *C. versicolor* is very similar in general to the enzyme from the brown-rot fungus *Tyromyces palustris*<sup>5)</sup>. (2) Formate dehydrogenase was cell-free extracted for the first time and detailed results will be reported elsewhere. (3) Oxalate biosynthesized by *C. versicolor* is in part mineralized to CO<sub>2</sub> according to the following metabolic pathway :



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